FEBS Letters 582 (2008) 773–777

Angiotensin II and III suppress food intake via angiotensin AT_2 receptor and prostaglandin EP_4 receptor in mice

Kousaku Ohinata[a], Yoko Fujiwara[a], Shingo Fukumoto[a], Masaru Iwai[b], Masatsugu Horiuchi[b], Masaaki Yoshikawa[a,*]

[a] *Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan*
[b] *Department of Molecular Cardiovascular Biology and Pharmacology, Ehime University Graduate School of Medicine, Shitsukawa, Tohon, Ehime 791-0295, Japan*

Received 5 September 2007; revised 19 December 2007; accepted 25 January 2008

Available online 5 February 2008

Edited by Laszlo Nagy

Abstract Intracerebroventricularly administered angiotensin (Ang) II and III dose-dependently suppressed food intake in mice and their anorexigenic activities were inhibited by AT_2 receptor-selective antagonist. Ang II did not suppress food intake in AT_2 receptor-knockout mice, while it did significantly in wild-type and AT_1 receptor-knockout mice. The suppression of food intake in AT_1 receptor-knockout mice was smaller than that in wild-type. The anorexigenic activities of Ang II and III were also blocked by a selective antagonist for prostaglandin EP_4 receptor. Taken together, centrally administered Ang II and III may decrease food intake through AT_2 receptor with partial involvement of AT_1 receptor, followed by EP_4 receptor activation, which is a novel pathway regulating food intake.

Keywords: Angiotensin II; Angiotensin AT_2 receptor; Food intake; Prostaglandin E_2; EP_4 receptor; Central nervous system

1. Introduction

The renin–angiotensin system (RAS) plays an important role in the regulation of blood pressure and fluid volume. It has been revealed that all the components of RAS, including angiotensinogen, enzymes responsible for releasing angiotensins (Ang) and Ang receptors, are present in the central nervous system (CNS) as well as the peripheral endocrine system [1,2]. Among the main bioactive peptides of central RAS, Ang II shows affinities for both AT_1 and AT_2 receptors, which are G-protein coupled seven-transmembrane receptors, while Ang III is slightly more selective for AT_2 receptor than Ang II [2–6]. It is known that the central administration of Ang II and III increases blood pressure, water intake, salt appetite and hormone release from the pituitary, and these activities are mediated by AT_1 receptor [2,7,8]. It has been reported that Ang II suppresses food intake after central infusion [9–11]; however, it has not been clear which receptor mediates the anorexigenic activity of Ang II. In the current study, we found that Ang III also suppresses food intake after centrally single administration. The potency of anorexigenic activities of Ang II and III was consistent with their affinity for AT_2 receptor. We thus investigated whether Ang II- and III-induced anorexigenic activities were mediated by AT_2 receptor using an AT_2 receptor-selective antagonist or AT_2 receptor-knockout mice.

We also investigated the mechanism of anorexigenic activities of Ang II and III downstream of AT_2 receptor. Prostaglandin (PG) E_2, a bioactive lipid produced in the CNS of mammals including humans, has a variety of physiologically and pathophysiologically central actions on wakefulness, fever, pain response and food intake [12–16]. PGE_2 exerts its actions through four different types of G-protein-coupled seven-transmembrane receptors, known as EP_1, EP_2, EP_3 and EP_4 receptors [17,18]. For example, EP_3 and EP_4 receptors mediate the febrile response and wakefulness effect of PGE_2, respectively [13,14]. We have very recently demonstrated that EP_4 receptor activation inhibits food intake in mice [19,20]. In tissue culture derived from the kidney, Ang II was reported to stimulate PGE_2 biosynthesis [21,22]. We then investigated whether Ang II- and III-induced anorexigenic activities were coupled to EP_4 receptor.

2. Materials and methods

2.1. Reagents

Angiotensin II and III were obtained from the Peptide Institute, Inc. (Osaka, Japan). An AT_2 receptor-selective antagonist PD123319 was purchased from Sigma–Aldrich, Co. (St. Louis, MO). An AT_1 receptor-selective antagonist CV-11974 (candesartan) and EP_4 receptor antagonist ONO-AE3-208 were kindly provided by Takeda Pharmaceutical Co., Ltd. (Osaka, Japan) and Ono Pharmaceutical Co., Ltd. (Osaka Japan), respectively [19,23].

2.2. Cannula implantation

Male ddY mice at 7 weeks of age were obtained from Japan SLC (Shizuoka, Japan). Male AT_{1a} receptor-deficient [24], AT_2 receptor-deficient [25] and wild-type C57BL/6J (Clea Japan, Inc., Tokyo, Japan) mice at 27 weeks of age were also used. Each mouse was individually housed under regulated conditions (22–24 °C on a 12 h light–dark cycle with lights on at 7 a.m.). Mice had free access to food pellets and water unless otherwise indicated. Intracerebroventricular (i.c.v.) administration was performed as previously described [19,20,26–28]. Briefly, mice were anesthetized with sodium pentobarbital (80–85 mg/kg i.p.) and placed in a stereotaxic instrument. A 24-gauge cannula beveled at one end over a distance of 3 mm (Safelet-Cas, Nipro, Osaka, Japan) was implanted 0.9 mm posterior to the bregma and 0.9 mm lateral to the suture for administration into the third cerebral ventricle. Animals were tested one week or more after implantation.

*Corresponding author. Fax: +81 774 38 3774.
E-mail address: yoshikawa@kais.kyoto-u.ac.jp (M. Yoshikawa).

0014-5793/$34.00
doi:10.1016/j.febslet.2008.01.054

2.3. Food intake

The food intake experiment was performed as previously described [19,20,26–28]. Angiotensin II or III was dissolved in artificial cerebrospinal fluid (ACSF; 138.9 mM NaCl, 3.4 mM KCl, 1.3 mM $CaCl_2$, 4.0 mM $NaHCO_3$, 0.6 mM NaH_2PO_4, 5.6 mM glucose, pH 7.4). The test started at 11 a.m. Mice fasted for 18 h with free access to water were i.c.v. administered with Ang II or Ang III in 4 μl ACSF or vehicle alone. AT_1 receptor-selective antagonist CV-11974 at a dose of 1 nmol/mouse was i.c.v. co-administrated with Ang II (1 nmol/mouse) in 4 μl ACSF. We confirmed that centrally administered Ang II (1 nmol/mouse)-induced water intake, which was mainly mediated by AT_1 receptor, was significantly blocked by 1 nmol/mouse of CV-11974 (data not shown), similarly to another AT_1 receptor antagonist valsartan as previously described [30]. AT_2 receptor antagonist PD123319 at a dose of 6.5 nmol/mouse [30] in 2 μl ACSF was administered 30 min before the central administration of Ang II or III (1 nmol/mouse) in 2 μl ACSF. The weight of the food pellets in each cage was measured at 0 and 20 min and 1, 2 h after i.c.v. administration of Ang II or III, and the cumulative food intake was calculated. All experiments were approved by Kyoto University Ethics Committee for Animal Research Use. All mice were killed by an overdose of anesthesia drugs after the experiment.

2.4. Statistical analysis

Values are expressed as the means ± S.E.M. Analysis of variance (ANOVA) followed by Fisher's test was used to assess differences among groups. *P* values less than 0.05 were considered significant.

3. Results

3.1. Centrally administered angiotensin II and III suppress food intake

Ang II dose-dependently suppressed food intake at a dose of 0.1–1 nmol/mouse 20 min after i.c.v. administration in male ddY mice fasted for 18 h, and the anorexigenic effect lasted for 60 min (Fig. 1a). Ang III also decreased food intake at a dose of 0.3–10 nmol/mouse 60 min after central administration in a dose-dependent manner (Fig. 1b). Ang III was reported to have 10-fold or 3-fold less potent affinity for AT_1 receptor or AT_2 receptor, respectively, than Ang II [6]. The minimum effective dose for the anorexigenic effect of Ang II (0.1 nmol/mouse) 20 min after administration was approximately one-third of Ang III (0.3 nmol/mouse) 60 min after administration. Ten nmol/mouse of Ang IV was inactive under our experimental condition (Fig. 1c). These results raise the possibility that AT_2 receptor activations might induce the suppression of food intake.

3.2. Anorexigenic activities of angiotensin II and III are mainly mediated by AT_2 receptor

We investigated whether Ang II- and III-induced anorexigenic effects are mediated by AT_2 or AT_1 receptor using their receptor-selective antagonists. AT_2 receptor-selective antagonist PD123319 (6.5 nmol/mouse, i.c.v.) significantly ($P < 0.01$) inhibited two-third of the anorexigenic activity of Ang II (1 nmol/mouse, i.c.v.) in male ddY mice, as shown in Fig. 2b. On the other hand, AT_1 receptor antagonist CV-11974 (1 nmol/mouse, i.c.v.) tended to block the anorexigenic activity of Ang II (1 nmol/mouse, i.c.v.) ($P = 0.134$, control vs Ang II with CV-11974); however, this was not significant ($P = 0.117$, Ang II with vs without CV-11974) (Fig. 1a). These results suggest that the anorexigenic activity of Ang II, which shows affinity for both AT_1 and AT_2 receptors, is mainly mediated by AT_2 receptor, though the contribution of AT_1 receptor is unclear. The anorexigenic effect of Ang III (1 nmol/mouse, i.c.v.), which was more selective for AT_2 receptor than Ang II, was completely blocked by PD123319 (6.5 nmol/mouse, i.c.v.) (Fig. 2c), suggesting that Ang III suppresses food intake via AT_2 receptor.

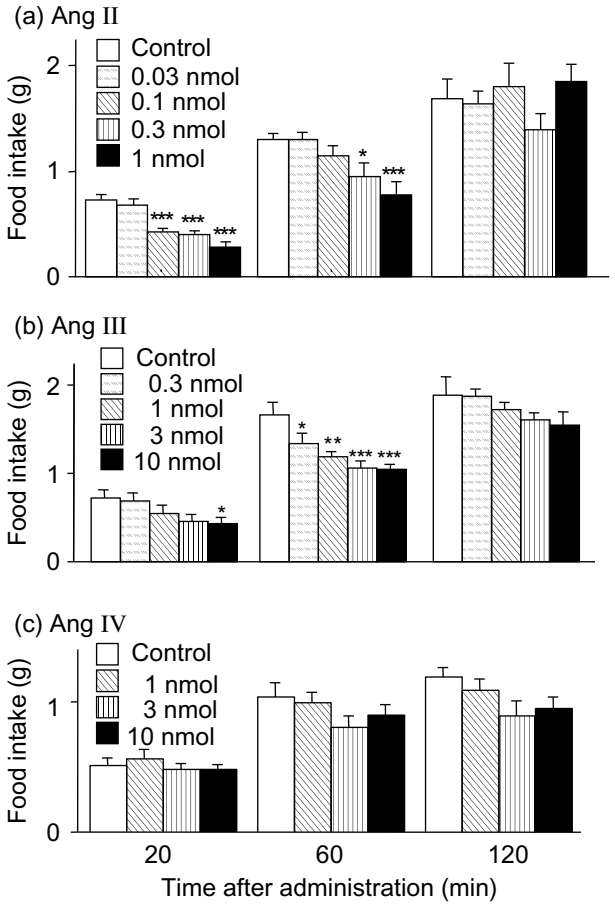

Fig. 1. Effect of central administration of angiotensins (Angs) on food intake in conscious male ddY mice fasted for 18 h. (a) Ang II (0.03–1 nmol/mouse), (b) Ang III (0.3–10 nmol/mouse) and Ang IV (1–10 nmol/mouse) were i.c.v. administered, and cumulative food intake was measured. Values are the means ± S.E.M. (a, $n = 7$–8; b, $n = 5$–7; c, $n = 7$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the ACSF control group.

To determine contribution of AT_2 and AT_1 receptors to the Ang II-induced anorexigenic activity, we also used the AT_2 and AT_1 receptors-knockout and wild-type C57BL/6 J mice. Ang II (1 nmol/mouse) did not suppress food intake 60 min after i.c.v. administration in AT_2 receptor-knockout mice, while did it in AT_1 receptor-knockout and wild-type mice (Fig. 3). However, the suppression of food intake by Ang II in AT_1 receptor-knockout mice was significantly smaller than that in wild-type mice, which was consistent with effect of AT_1 antagonist, suggesting that Ang II-induced anorexigenic activity is partly mediated through AT_1 receptor. Taken together, AT_2 receptor activation led to suppression of food intake by Ang II with partial involvement of AT_1 receptor.

3.3. Anorexigenic activities of angiotensin II and III are mediated by prostaglandin E_2-EP_4 receptor

Centrally administered PGE_2 suppresses food intake via EP_4 receptor among four subtypes for PGE_2. The anorexigenic

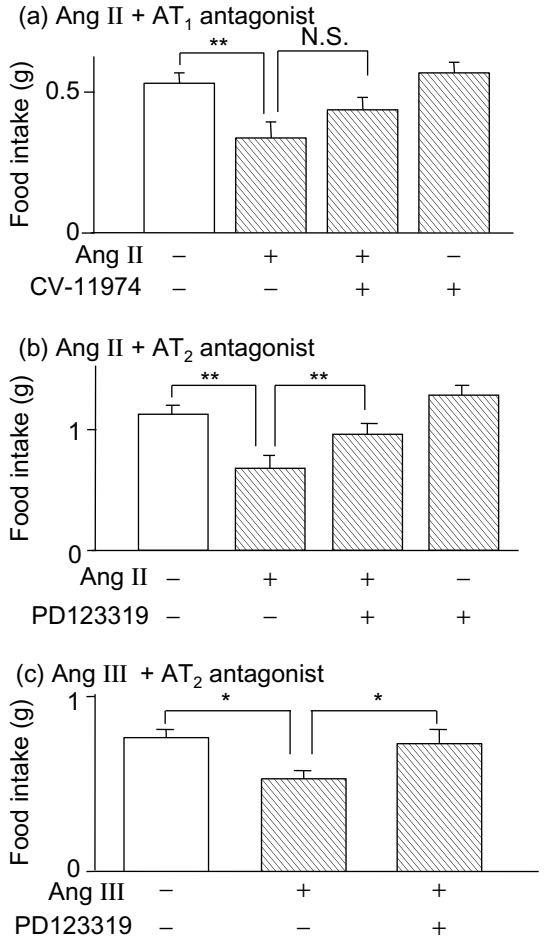

Fig. 2. Effect of a selective antagonist for AT_1 or AT_2 receptor on the anorexigenic activity of Ang II or III after central administration. (a) AT_1 receptor-selective antagonist CV-11974 (1 nmol/mouse) or (b and c) AT_2 receptor-selective antagonist PD123319 (6.5 nmol/mouse) was i.c.v. administered with (a and b) Ang II or (c) Ang III at a dose of 1 nmol/mouse in ddY mice fasted for 18 h. Food intake was measured for (a and c) 20 min and (b) 60 min. Values are presented as the means ± S.E.M. (n = 7–8). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the control group.

activities of Ang II and III (1 nmol/mouse, i.c.v.) were blocked by an EP_4 receptor-selective antagonist ONO-AE3-208 (10 nmol/mouse, i.c.v.) in fasted ddY mice (Fig. 4a and b). Taken together, we demonstrated that Ang II and III decreased food intake via PGE_2 production and EP_4 receptor activation.

4. Discussion

We found that not only Ang II but also Ang III suppress food intake after central administration to fasted mice. The affinities of Ang III and II for the AT_2 receptor are consistent with their anorexigenic activities. Blockade of the AT_2 receptor using a selective antagonist and knockout mice abolished the suppression of food intake of centrally administered Ang agonist peptides. We thus demonstrated for the first time that activation of the central AT_2 receptor induced food intake suppression.

The Ang system, including angiotensin I-converting enzyme (ACE), and Ang II and Ang receptors (AT_1 and AT_2 receptors), are localized in the hypothalamus in the CNS [1,2,29], which is known to play important roles in not only regulating food intake but also water intake and blood pressure [1,2]. The AT_2 receptor, which mediates anorexigenic activities of Ang agonist peptides such as Ang II and III, was reported to be present in the paraventricular nucleus (PVN) of the hypothalamus using in situ autoradiography [29]. AT_1 receptor, which

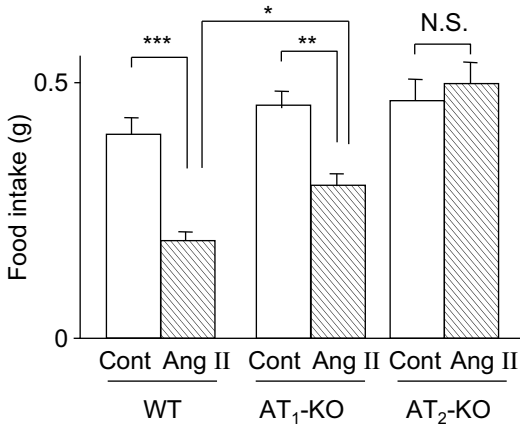

Fig. 3. Suppression of food intake in wild-type C57BL/6 J, AT_1 receptor-knockout and AT_2 receptor-knockout mice. Each male mouse fasted for 18 h was i.c.v. administered with Ang II at a dose of 1 nmol/mouse, and food intake was measured for 60 min. Values are presented as the means ± S.E.M. (n = 8–17). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the control group.

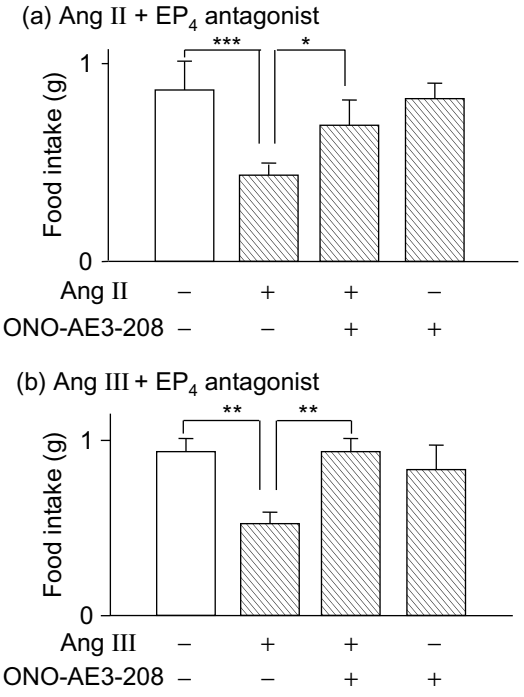

Fig. 4. Effect of an EP_4 receptor antagonist on the anorexigenic activity of Ang II or III. An EP_4 receptor-selective antagonist ONO-AE3-208 (10 nmol/mouse, i.c.v.) was administered 30 min before Ang II or III (1 nmol/mouse, i.c.v.) in male ddY mice fasted for 18 h, and food intake was measured for 60 min. Values are presented as the means ± S.E.M. (n = 6–7). $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$ compared with each group.

is mainly associated with water intake, blood pressure regulation and vasopressin secretion [2], was detected in the PVN, suprachiasmatic nucleus, and median eminence of the hypothalamus in the CNS [29].

AT_1 receptor often functionally interacts with AT_2 receptor in the CNS. For example, centrally administered Ang II increased systolic blood pressure in wild-type and AT_2 receptor-knockout mice; however, this increase was significantly greater in AT_2-knockout mice than in WT mice [30]. On the other hand, stimulation of water intake-induced by Ang II was partly suppressed in both AT_1 receptor- and AT_2 receptor-knockout mice, although the suppressive effect in AT_1-knockout mice was more potent than that in AT_2 receptor-knockout mice. These results indicate that central AT_1 and AT_2 receptors act reversely each other in blood pressure regulation, while they act synergistically in water intake regulation [30]. In the current study, centrally administered Ang II suppressed food intake in wild-type and AT_1 receptor-knockout mice but not in AT_2 receptor-knockout mice; however, the anorexigenic activity of Ang II in AT_1 receptor-knockout mice less potent than that in wild-type mice. These results suggest that AT_1 receptor partly mediates Ang II-induced anorexigenic activity, though its contribution is smaller than that of AT_2 receptor. Thus, the anorexigenic activities of Ang II after central administration may be mainly mediated via AT_2 receptor with partial involvement of AT_1 receptor.

We also found that an AT_2 receptor-selective agonist peptide novokinin [31], which we designed as an orally active hypotensive peptide, decreased food intake both in wild-type and AT_1 receptor-knockout mice to the same extent, while it was inactive in AT_2 receptor-knockout mice (unpublished data). This suggests that the anorexigenic activity of novokinin is mediated by AT_2 receptor.

We have recently demonstrated that the activation of EP_4 receptor among PGE_2 receptors inhibits food intake in mice [19,20]; however, the pathways upstream of EP_4 receptor have been unclear. Anorexigenic activities of Ang II and III after central administration were inhibited by EP_4 receptor, indicating that AT_2 receptor is coupled to the PGE_2-EP_4 receptor system. In the hypothalamus, EP_4 receptor mRNA was abundantly localized in the PVN and the supraoptic nucleus [17,32], where the AT_2 receptor also exists [29]. PGE_2 is produced from arachidonic acid by cyclooxygenase (COX) followed by PGE synthase, and acts near to its production site [17,33]. It was reported that COX and PGE synthase are constitutively present in the PVN [33]. In the current study, we demonstrated that the PGE_2-EP_4 receptor was coupled downstream of AT_2 receptor, and this signal transduction is a novel pathway regulating food intake in the CNS.

It has recently been reported that some AT_1 antagonists by themselves suppress food intake [34]. After AT_1 receptor antagonist administration, endogenous Ang II, having affinities for both AT_1 and AT_2 receptors, might act as an AT_2 receptor agonist. From our results, this AT_2 receptor activation might be one explanation for the anorexigenic activities of AT_1 receptor antagonists.

In conclusion, we found that exogenously administered Ang II and III decrease food intake after central administration. Blockages of angiotensin AT_2 receptor using a selective antagonist and knockout mice abolished the anorexigenic activities of centrally administered Ang II and III. These results suggest that activation of central AT_2 receptor suppresses food intake. Furthermore, the Ang II- and III-induced anorexigenic actions were inhibited by an EP_4 receptor antagonist. Taken together, the AT_2 receptor might be coupled to the PGE_2-EP_4 receptor system in food intake regulation in the CNS.

Acknowledgements: This work was supported, in part, by Grants-in-Aid for Scientific Research from Japanese Society for Promotion of Science to M.Y. and K.O., and a PROBRAIN Grant from the Bio-oriented Technology Research Advancement Institution to M.Y.

References

[1] von Bohlen und Halbach, O. and Albrecht, D. (2006) The CNS renin–angiotensin system. Cell Tissue Res. 326 (2), 599–616, (Epub 2006 Mar 23, Review).
[2] Reaux, A., Fournie-Zaluski, M.C. and Llorens-Cortes, C. (2001) Angiotensin III: a central regulator of vasopressin release and blood pressure. Trends Endocrinol. Metab. 12 (4), 157–162, (Review)..
[3] Murphy, T.J., Alexander, R.W., Griendling, K.K., Runge, M.S. and Bernstein, K.E. (1991) Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. Nature 351 (6323), 233–236.
[4] Kambayashi, Y., Bardhan, S., Takahashi, K., Tsuzuki, S., Inui, H., Hamakubo, T. and Inagami, T. (1993) Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. J. Biol. Chem. 268 (33), 24543–24546.
[5] Mukoyama, M., Nakajima, M., Horiuchi, M., Sasamura, H., Pratt, R.E. and Dzau, V.J. (1993) Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors. J. Biol. Chem. 268 (33), 24539–24542.
[6] Rosenstrom, U., Skold, C., Lindeberg, G., Botros, M., Nyberg, F., Hallberg, A. and Karlen, A. (2004) Synthesis and AT2 receptor-binding properties of angiotensin II analogues. J. Pept. Res. 64 (5), 194–201.
[7] Wright, J.W. and Harding, J.W. (1997) Important role for angiotensin III and IV in the brain renin–angiotensin system. Brain Res. Brain Res. Rev. 25 (1), 96–124, (Review).
[8] Saavedra, J.M. (2005) Brain angiotensin II: new developments, unanswered questions and therapeutic opportunities. Cell Mol. Neurobiol. 25 (3–4), 485–512.
[9] Gronan, R.J. and York, D.H. (1979) Effects of chronic intraventricular administration of angiotensin II on drinking behavior and blood pressure. Pharmacol. Biochem. Behav. 10 (1), 121–126.
[10] Porter, J.P., Anderson, J.M., Robison, R.J. and Phillips, A.C. (2003) Effect of central angiotensin II on body weight gain in young rats. Brain Res. 959 (1), 20–28.
[11] Porter, J.P. and Potratz, K.R. (2004) Effect of intracerebroventricular angiotensin II on body weight and food intake in adult rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287 (2), R422–R428.
[12] Hayaishi, O. (1991) Molecular mechanisms of sleep–wake regulation: roles of prostaglandins D_2 and E_2. FASEB J. 5 (11), 2575–2581.
[13] Ushikubi, F., Segi, E., Sugimoto, Y., Murata, T., Matsuoka, T., Kobayashi, T., Hizaki, H., Tuboi, K., Katsuyama, M., Ichikawa, A., Tanaka, T., Yoshida, N. and Narumiya, S. (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. Nature 395 (6699), 281–284.
[14] Huang, Z.L., Sato, Y., Mochizuki, T., Okada, T., Qu, W.M., Yamatodani, A., Urade, Y. and Hayaishi, O. (2003) Prostaglandin E_2 activates the histaminergic system via the EP_4 receptor to induce wakefulness in rats. J. Neurosci. 23 (14), 5975–5983.
[15] Horton, E.W. (1964) Actions of prostaglandins E1, E2 and E3 on the central nervous system. Br. J. Pharmacol. 22, 189–192.
[16] Levine, A.S. and Morley, J.E. (1981) The effect of prostaglandins (PGE_2 and PGF_2 alpha) on food intake in rats. Pharmacol. Biochem. Behav. 15 (5), 735–738.
[17] Narumiya, S., Sugimoto, Y. and Ushikubi, F. (1999) Prostanoid receptors: structures, properties, and functions. Physiol. Rev. 79 (4), 1193–1226.

[18] Narumiya, S. and FitzGerald, G.A. (2001) Genetic and pharmacological analysis of prostanoid receptor function. J. Clin. Invest. 108 (1), 25–30.
[19] Ohinata, K., Suetsugu, K., Fujiwara, Y. and Yoshikawa, M. (2007) Suppression of food intake by a complement C3a agonist [Trp^5]-oryzatensin(5-9). Peptides 28 (3), 602–606.
[20] Ohinata, K., Suetsugu, K., Fujiwara, Y. and Yoshikawa, M. (2006) Activation of prostaglandin E receptor EP_4 subtype suppresses food intake in mice. Prostaglandins Other Lipid Mediat. 81 (1–2), 31–36.
[21] Zusman, R.M. and Keiser, H.R. (1977) Prostaglandin E_2 biosynthesis by rabbit renomedullary interstitial cells in tissue culture. Mechanism of stimulation by angiotensin II, bradykinin, and arginine vasopressin. J. Biol. Chem. 252 (6), 2069–2071.
[22] Ferreri, N.R., Zhao, Y., Takizawa, H. and McGiff, J.C. (1997) Tumor necrosis factor-alpha-angiotensin interactions and regulation of blood pressure. J. Hypertens. 15 (12 Pt. 1), 1481–1484.
[23] Suzawa, T., Miyaura, C., Inada, M., Maruyama, T., Sugimoto, Y., Ushikubi, F., Ichikawa, A., Narumiya, S. and Suda, T. (2000) The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. Endocrinology 141 (4), 1554–1559.
[24] Sugaya, T., Nishimatsu, S., Tanimoto, K., Takimoto, E., Yamagishi, T., Imamura, K., Goto, S., Imaizumi, K., Hisada, Y., Otsuka, A., Uchida, H., Sugiura, M., Fukuta, K., Fukamizu, A. and Murakami, K. (1995) Angiotensin II type 1a receptor-deficient mice with hypotension and hyperreninemia. J. Biol. Chem. 270 (32), 18719–18722.
[25] Hein, L., Barsh, G.S., Pratt, R.E., Dzau, V.J. and Kobilka, B.K. (1995) Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor in mice. Nature 377 (6551), 744–747.
[26] Ohinata, K., Inui, A., Asakawa, A., Wada, K., Wada, E. and Yoshikawa, M. (2002) Albutensin A and complement C3a decrease food intake in mice. Peptides 23 (1), 127–133.
[27] Ohinata, K., Shimano, T., Yamauchi, R., Sakurada, S., Yanai, K. and Yoshikawa, M. (2004) The anorexigenic effect of neurotensin is mediated via a histamine H1 receptor in mice. Peptides 25 (12), 2135–2138.
[28] Ohinata, K., Inui, A., Asakawa, A., Wada, K., Wada, E. and Yoshikawa, M. (2001) Proadrenomedullin N-terminal 20 peptide (PAMP) inhibits food intake and gastric emptying in mice. Peptides 22 (4), 589–595.
[29] Jöhren, O., Imboden, H., Häuser, W., Maye, I., Sanvitto, G.L. and Saavedra, J.M. (1997) Localization of angiotensin-converting enzyme, angiotensin II, angiotensin II receptor subtypes, and vasopressin in the mouse hypothalamus. Brain Res. 757 (2), 218–227.
[30] Li, Z., Iwai, M., Wu, L., Shiuchi, T., Jinno, T., Cui, T.X. and Horiuchi, M. (2003) Role of AT_2 receptor in the brain in regulation of blood pressure and water intake. Am. J. Physiol. Heart Circ. Physiol. 284 (1), H116–H121.
[31] Yamada, Y., Yamauchi, D., Usui, H., Yokoo, M., Ohinata, M., Iwai, M., Horiuchi, M., Yoshikawa, M. Hypotensive activity of novokinin, a potent analogue of ovokinin(2-7), is mediated by angiotensin AT_2 receptor and prostaglandin IP receptor. Peptide (in press).
[32] Zhang, J. and Rivest, S. (1999) Distribution, regulation and colocalization of the genes encoding the EP2- and EP4-PGE2 receptors in the rat brain and neuronal responses to systemic inflammation. Eur. J. Neurosci. 11 (8), 2651–2668.
[33] Matsuoka, Y., Furuyashiki, T., Bito, H., Ushikubi, F., Tanaka, Y., Kobayashi, T., Muro, S., Satoh, N., Kayahara, T., Higashi, M., Mizoguchi, A., Shichi, H., Fukuda, Y., Nakao, K. and Narumiya, S. (2003) Impaired adrenocorticotropic hormone response to bacterial endotoxin in mice deficient in prostaglandin E receptor EP1 and EP3 subtypes. Proc. Natl. Acad. Sci. USA 100 (7), 4132–4137.
[34] Voigt, J.P., Bramlage, P. and Fink, H. (2007) Hypophagic effect of the angiotensin AT_1 receptor antagonist irbesartan in rats. Eur. J. Pharmacol. 564 (1–3), 131–137.